CYFRA 21-1 as a Prognostic and Predictive Marker in Advanced Non–Small-Cell Lung Cancer in a Prospective Trial: CALGB 150304

Martin J. Edelman, MD, Lydia Hodgson, MS,† Paula Y. Rosenblatt, MD,* Robert H. Christenson, PhD,* Everett E. Vokes, MD,§ Xiaofei Wang, PhD,† and Robert Kratzke, MD,‡ for the Cancer and Leukemia Group B*

Background: Cytokeratin 19 and its soluble fragment CYFRA have been studied as markers that may be associated with response to therapy and survival in non–small-cell lung cancer (NSCLC). As a prospective correlative study of Cancer and Leukemia Group B 30203, a randomized phase II trial of carboplatin/gemcitabine with eicosanoid modulators (celecoxib, zileuton, or both) in advanced NSCLC, serum CYFRA levels were obtained before and during treatment.

Methods: Serum CYFRA levels were measured at baseline and after the first cycle of treatment using an electrochemoluminescent assay. Paired specimens were available from 88 patients. The logarithms of the initial concentration and of the difference in concentrations were analyzed for association with overall survival (OS) and failure-free survival (FFS).

Results: Lower baseline CYFRA levels were associated with both longer OS and FFS ($p < 0.0001$ and $p = 0.0003$). In addition, larger reductions in CYFRA levels correlated with longer OS and FFS ($p = 0.0255$ and $p = 0.0068$).

Conclusion: CYFRA and change in CYFRA were found to be reliable markers for response to chemotherapy for NSCLC; however, a precise threshold to mark response has yet to be determined.

Key Words: Lung cancer, CYFRA.

(*J Thorac Oncol.* 2012;7: 649–654)

Biologic markers have become essential in guiding the treatment of many cancers, such as prostate and ovarian cancer. Identification of these markers saves time, money, and radiation exposure. Various markers such as carcinoembryonic antigen, neuron-specific enolase, tissue polypeptide specific antigen, squamous cell carcinoma antigen, and cancer antigen 125 have been studied in terms of their prognostic or predictive implications in lung cancer or as a method of assessing response to therapy.[1,2] However, to date, no serum marker is currently recommended for routine clinical practice in non–small-cell lung cancer (NSCLC). One of the most promising markers for NSCLC is the soluble fragment of cytokeratin 19. Simple epithelium, such as bronchial epithelium, is composed of intermediate filaments that give the cell its structure and strength. In malignant tissues, the intermediate filament known as cytokeratin 19 and the C-terminus of cytokeratin 19 (CYFRA 21-1, CYFRA) are released into circulation by a cleaving enzyme, caspase-3, and apoptosis.[3] For almost two decades, research has evaluated whether the serum levels of these filaments may relate to prognosis. In 2003, Vollmer et al[4] summarized the research done before 1999 and reported a trial completed at four Cancer and Leukemia Group B (CALGB) institutions evaluating the levels of CYFRA in 58 patients with stage III and IV NSCLC treated with chemotherapy. In this study, higher initial CYFRA concentrations predicted a worse prognosis and the ratio of logarithm of CYFRA before and after one cycle of chemotherapy correlated with prognosis. Both the initial natural logarithm of serum CYFRA and the presence of a less than 27% drop in CYFRA were significantly related to subsequent survival. As part of a prospective CALGB study evaluating chemotherapy and eicosanoid modulation in advanced NSCLC (CALGB 30203), we sought to confirm these findings. The evaluation of CYFRA, including the statistical objectives, was prospectively defined in the protocol.

PATIENTS AND METHODS

CALGB 30203 tested the concept of eicosanoid inhibition in advanced lung cancer and has been previously reported.[5] The hypothesis was that eicosanoid inhibition in addition to standard chemotherapy would potentially increase progression-free survival. Furthermore, the concept of single versus double pathway inhibition was tested with inhibitors of cyclooxygenase-2 and 5-lipoxygenase as both single agents and in combination. Patients with advanced NSCLC (stage IIIB [pleural effusion]/IV) with performance status (PS) 0 to 2, and normal organ function were randomized to receive chemotherapy (carboplatin area under the curve = 5.5, day 1 and gemcitabine 1000 mg/m^2 day 1, 8) with one of three eicosanoid modulating regimens: zileuton 600 mg four times

*University of Maryland Greenebaum Cancer Center, Baltimore, Maryland; †Duke University, Durham, North Carolina; ‡University of Minnesota, Minneapolis, Minnesota; and §University of Chicago, Chicago, Illinois.

Disclosure: The authors declare no conflict of interest.

Address for correspondence: Martin J. Edelman, MD, University of Maryland Greenebaum Cancer Center, 22 S. Greene Street, Baltimore, MD 21201. E-mail: medelman@umm.edu

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ISSN: 1556-0864/12/0704-0649

a day, celecoxib 400 mg two times a day, or both agents. Each participant signed an Institutional Review Board-approved, protocol-specific informed consent in accordance with Federal and institutional guidelines.

To evaluate CYFRA levels, blood was collected in a 7-ml red top tube, inverted 5 times, left at room temperature to clot for 30 minutes, and then spun at 1100 to 1330 g for 10 minutes in a swinging head rotor at 25°C. Serum was then removed and placed in a polypropylene tube and frozen to −20°C or colder. Shipment to the CALGB Pathology Coordinating Office was done on dry ice. Analysis of CYFRA levels was conducted on specimens at first thaw. CYFRA levels in the serum were measured by using two monoclonal antibodies to sandwich the molecule, KS 19.1 and BM 19.21. One antibody was labeled with a Ruthenium complex, which is electrochemically luminescent, and the other with a magnetic particle. When the electric potential is applied to the molecules, light is produced and measured by a photomultiplier. The coefficient of variation is 2 to 5%.[2] Samples were analyzed at the University of Maryland by Dr. Christenson in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory without knowledge of patient characteristics or outcomes.

Patient registration and clinical data were managed by the CALGB Statistical Center. The statistical analysis was performed at the CALGB Statistical Center. The balance of demographic and clinical variables across study arms was tested by χ^2 tests for categorical variables and Wilcoxon rank sum tests for continuous variables. Kaplan-Meier curves were used to characterize overall survival (OS) and failure-free survival (FFS), in which OS was defined as the time from study entry to the date of death resulting from any cause, and FFS was defined as the time from study entry to the date of disease progression or death, whichever came first. Finally, Cox regression analysis was used to assess the association of baseline CYFRA and the change of cycle-1 CYFRA relative to the baseline with survival end points. The logarithmic concentration values of CYFRA were used as previous studies show them to have association with outcome and the logarithmic transformation also serves to reduce the influence of extreme CYFRA values. As the decrease of cycle-1 CYFRA value relative to its baseline is a posttreatment covariate, the survival end points (OS and FFS) for this analysis are redefined by starting time from the end date of cycle-1 chemotherapy.

RESULTS

CALGB 30203 enrolled 140 patients in less than 1 year and showed no difference in OS or FFS among the three arms. Adequate serum samples before therapy and after the first cycle were obtained for 88 of the 140 patients (63%). Table 1 shows patient characteristics of those patients in CALGB 30203 who had CYFRA concentrations analyzed. There were no significant differences among the arms, and the population for which samples were obtained for this analysis was comparable to the entire study population.

Table 2 shows the median, mean, minimum, and maximum CYFRA levels in all three arms at baseline and after cycle 1 of chemotherapy. Baseline CYFRA levels ranged from 0.44 to 204.2 ng/ml with a median CYFRA level of 4.18 ng/ml and a mean level of 12.9 ng/ml. A Kaplan-Meier survival plot was constructed comparing those with initial CYFRA levels

TABLE 1. Baseline Patient Characteristics of the 88 Patients with Serum CYFRA Levels Analyzed

Characteristics	CYFRA Decrease ≤ 27% (n = 47)	CYFRA Decrease >27% (n = 41)	Total (n = 88)
Sex			
Male	26 (55%)	28 (68%)	54 (61%)
Female	21 (45%)	13 (32%)	34 (39%)
Age (yr)			
<60	19 (40%)	21 (51%)	40 (46%)
60–69	16 (34%)	14 (34%)	30 (34%)
⩾70	12 (26%)	6 (15%)	18 (21%)
Median (min, max)	61 (41, 80)	59 (49, 81)	60 (41, 81)
Race			
White	38 (81%)	37 (90%)	75 (85%)
Black or other	9 (19%)	4 (10%)	13 (15%)
Histology			
Adenocarcinoma	25 (53%)	19 (46%)	44 (50%)
Squamous	12 (26%)	9 (22%)	21 (24%)
Undifferentiated	10 (21%)	13 (32%)	23 (26%)
Performance status			
0	14 (30%)	13 (32%)	27 (31%)
1 or 2	33 (70%)	28 (68%)	61 (69%)
Stage			
IIIB	5 (11%)	2 (5%)	7 (8%)
IV	39 (83%)	37 (90%)	76 (86%)
Recurrent	3 (6%)	2 (5%)	5 (6%)

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TABLE 2. CYFRA Concentrations (ng/ml)

	Median, Mean (minimum, maximum)		
	CYFRA Decrease ⩽ 27% (n = 47)	**CYFRA Decrease >27% (n = 41)**	**Total (n = 88)**
CYFRA baseline	3.7, 12.7 (0.72, 204.2)	4.7, 13.2 (0.44, 87.0)	4.2, 12.9 (0.44, 204.2)
Log CYFRA baseline	1.3, 1.5 (−0.33, 5.3)	1.5, 1.6 (−0.82, 4.5)	1.4, 1.6 (−0.82, 5.3)
CYFRA cycle 1	5.5, 10.4 (0.51, 54.4)	1.7, 3.2 (0.66, 13.6)	4.3, 7.1 (0.51, 54.4)
Log CYFRA cycle 1	1.7, 1.8 (−0.67, 4.0)	0.51, 0.83 (−0.42, 2.6)	1.5, 1.4 (−0.67, 4.0)
CYFRA baseline–CYFRA cycle 1	−1.0, 2.2 (−28.9, 154.4)	2.4, 10.0 (−0.87, 78.8)	0.53, 5.9 (−28.9, 154.4)
Log (CYFRA baseline)–Log (CYFRA cycle 1)	−0.30, −0.29 (−1.8, 1.4)	0.70, 0.77 (−0.89, 2.6)	0.21, 0.20 (−1.8, 2.6)

above and below the median. As shown in Figure 1, patients with initial CYFRA levels below the median had a statistically significant increase in their OS ($p = 0.0216$). After log transformation, higher baseline CYFRA correlated with worse OS and FFS ($p < 0.0001$ and $p = 0.003$) (Table 3).

After cycle 1, the median CYFRA level remained steady at 4.3 but the mean CYFRA level fell to 7.1 (Wilcoxon signed rank test, $p = 0.0225$, Table 2). After logarithmic transformation, a greater reduction in CYFRA from baseline to after cycle 1 correlated with longer overall and FFS ($p = 0.0255$ and $p = 0.0068$) in the multivariate analysis after adjusting for age and baseline CYFRA (Model 1, Table 3). We also confirmed the prognostic value of a greater than 27% decline in CYFRA being associated with better OS or FFS survival ($p = 0.0028$ and $p = 0.0074$) (Table 3). This decline occurred in 41 of the 88 patients tested. Our analysis also confirmed that a greater than 27% decline on a logarithmic scale is also the optimal cutoff point that yields the largest separation between the high versus low CYFRA-decline patients (Table 3). There were no statistically significant differences in CYFRA levels at cycle 1 (Wilcoxon rank sum test, $p = 0.3654$) or changes from baseline to cycle 1 (Wilcox rank sum test, $p = 0.7791$) related to the type of eicosanoid modulator employed. There was no relationship between a 27% decline in CYFRA and response ($p = 0.114$).

Multivariate analysis that included age, sex, PS, and staging (IIIB versus IV) was performed. Only age emerged as a significant factor. Data regarding smoking status were not collected. Logistic regression analysis (OR = 1.62, 95% confidence interval 1.09–2.40; $p = 0.0161$) indicated that squamous

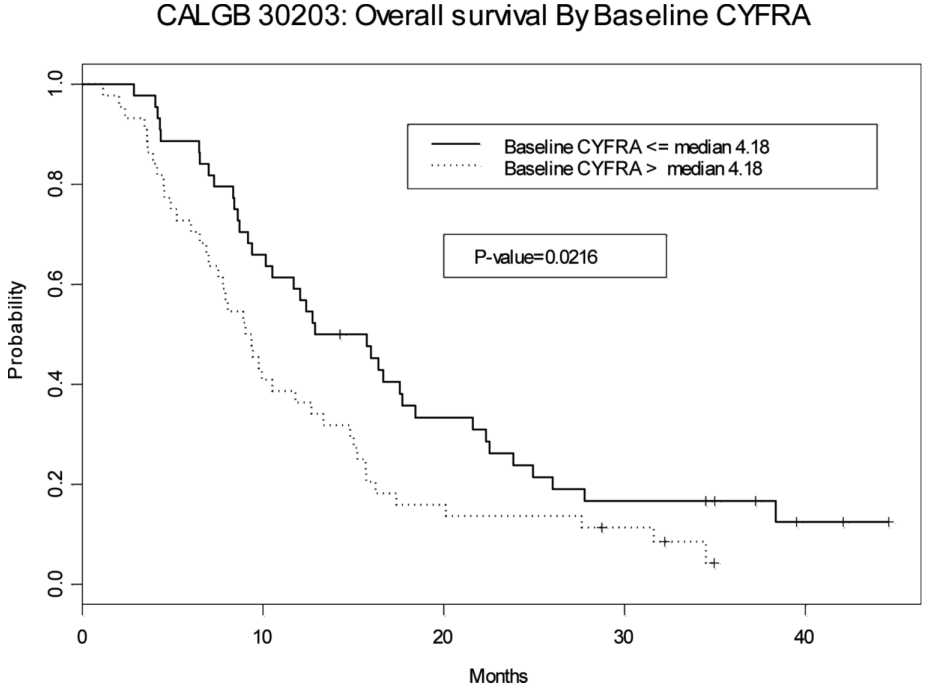

FIGURE 1. Kaplan-Meier survival curve based on baseline CYFRA above and below the median value.

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TABLE 3. Multivariate Survival Analysis on CYFRA Concentrations

Parameter	OS[a]		FFS[a]	
	p Value	Hazard Ratio 95% CI	*p* Value	Hazard Ratio 95% CI
Model 1[b]				
Log (baseline CYFRA)	<0.0001	1.68 (1.33, 2.11)	0.0003	1.43 (1.23, 2.04)
Log (baseline CYFRA)–Log (CYFRA cycle 1)	0.0255	0.73 (0.56, 0.96)	0.0068	0.66 (0.49, 0.89)
Age (> 65 vs. ≤65)	0.0176	1.81 (1.11, 2.95)	0.2360	1.33 (0.83, 2.15)
Model 2[c]				
Log (baseline CYFRA)	<0.0001	1.55 (1.27, 1.90)	0.0023	1.34 (1.10, 1.62)
27% or greater decline in CYFRA from baseline to cycle 1 (yes vs. no)	0.0028	0.49 (0.31, 0.78)	0.0074	0.52 (0.32, 0.84)
Age (>65 vs. ≤65 yr)	0.0152	1.83 (1.12, 2.98)	0.2407	1.33 (0.83, 2.14)

[a]The decrease of cycle 1 CYFRA relative to baseline is a posttreatment covariate; in this analysis, the survival end points, OS and FFS, were redefined to have time start at the end of cycle 1 chemotherapy.

[b]Model 1 is the final model of Cox proportional hazards regression analysis. Log baseline CYFRA and Log (baseline CYFRA)–Log (CYFRA cycle 1) were forced into the final model for OS with performance status, age (>65 vs. <65 years), treatment arm, sex, race, and histology as potential variables to be selected using stepwise algorithm with entry level of 0.10 and stay level of 0.10. The model for FFS is chosen to be the same as for OS.

[c]Model 2 is the final model chosen by similar variable selection procedure to Model 1. The predictor "27% or greater decline in Log CYFRA from baseline to cycle 1" is a binary variable with 1 denoting 27% or greater decline on logarithmic CYFRA from baseline to cycle 1. Vollmer et al[4] used a 27% or greater decline on raw CYFRA scale, but our analysis on their definition yields a less-significant association for OS ($p = 0.1002$) and FFS ($p = 0.1797$).

OS, overall survival; FFS, failure-free survival; CI, confidence interval.

patients had higher baseline CYFRA levels. However, there was no correlation between changes of CYFRA and histology (OR = 0.97, $p = 0.9068$). In addition, no correlation was noted between baseline CYFRA and sex ($p = 0.1146$), age ($p = 0.0635$), race ($p = 0.1088$), PS ($p = 0.1549$), histology ($p = 0.1512$), stage IIIB versus IV ($p = 0.0765$).

DISCUSSION

This study prospectively confirms in a multicenter trial that serum concentrations of CYFRA have prognostic value in advanced NSCLC. Higher baseline CYFRA concentrations portend worse OS and FFS. In addition, this trial confirmed the significance of the cut point of a 27% reduction in log CYFRA after chemotherapy in determining benefit from treatment. Other cut points, including 10 to 75% decline, worked almost equally well (data not shown).

This information may be useful in determining whether or not to continue a particular chemotherapy regimen. If confirmed, this would provide a simple and inexpensive approach to the assessment of response to treatment.

Of note, these findings are qualitatively similar to those of others as summarized by Vollmer et al (studies before 1999) and in Table 4 (studies after 1999).[4] Prior studies have found that CYFRA elevations have correlated with stage and predicted for recurrent disease after surgery and for inferior survival. The significance of the current trial is that the patients were part of a prospective multicenter trial employing standard entry criteria and a uniform chemotherapy regimen.

There are several limitations to the current study. The number of patients studied was relatively small. In addition, as all arms used eicosanoid modulation, the chemotherapy regimens could be considered "nonstandard." However, all patients received standard, platinum-based two-drug chemotherapy. In addition, both of the experimental agents studied, celecoxib and/or zileuton, are drugs that are commercially available and they (or similar drugs) are commonly prescribed for patients with lung cancer. Another potentially confounding factor is a possible difference in "bulk" of the disease, which was not clearly captured in the required data and for which there is no standardized approach. It is quite possible that CYFRA is a nonspecific marker for tumor burden. Furthermore, this study did not evaluate nor compare CYFRA to other possible serum markers that have been used, such as carcinoembryonic antigen.[6]

In the current landscape of markers for NSCLC, CYFRA is unlikely to have the strong prognostic or predictive value of epithelial growth factor receptor activating mutations or EML4/ALK translocations. However, it may ultimately find a role as an early marker of tumor responsiveness to therapy.

In summary, this study demonstrates the potential value of CYFRA 21-1 as both a prognostic marker in advanced NSCLC and an early indicator of response to chemotherapy. Further studies are warranted to compare the value of CYFRA to radiologic imaging in determining response.

ACKNOWLEDGMENTS

The research for CALGB 15034 was supported, in part, by grants from the National Cancer Institute (CA31946) to the Cancer and Leukemia Group B (Monica M. Bertagnolli, MD, Chair) and to the CALGB Statistical Center (Daniel J. Sargent, PhD, CA33601). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute.

The following institutions participated in this study: University of Oklahoma, Oklahoma, OK—Shubham Pant, MD, supported by CA37447; Christiana Care Health Services, Inc. CCOP, Wilmington, DE—Stephen Grubbs, MD, supported by CA45418; Duke University Medical Center,

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TABLE 4. Recent Studies of CYFRA in NSCLC from 1999 to 2009

Study	Stage	Findings
Localized disease		
Yeh et al[7]	Stages I–IIIA	Elevations in CYFRA after surgery predicted reoccurrence.
Muley et al[8]	Stage I	In postoperative–stage I patients, 3-yr survival was statistically shorter with elevated CYFRA 21-1 levels >3.3 ng/ml. Consideration given for these patients to receive adjuvant chemotherapy.
Suzuki et al[9]	Stage I	Elevated CYFRA 21-1 levels in early-stage operative NSCLC predicts poor outcome and should be evaluated for possible chemotherapy.
Advanced		
Vollmer et al[4]	Stage III/IV	Initial CYFRA level gives more prognostic information than stage. A decline of ⩾27% after one cycle of chemotherapy improves prognosis.
Barlési et al[10]	Stage IIIB/IV	CYFRA levels ⩾ 3.5 ng/ml correlated to poorer prognosis. CYFRA combined with carcinoembryonic antigen and neuron-specific enolase correlated with more accurate prognosis.
Merle et al[11]	Stage IIIB/IV	A drop of 80% in CYFRA after one cycle of chemotherapy was the most predictive of OS when compared with initial staging, tumor response, and surgery.
Ardizzoni et al[2]	Advanced	Patients with CYFRA declines of ⩾20% after two cycles had increased median survival of 5 months (6 months vs. 11 months).
Holdenrieder et al[12]	Advanced	Slower and incomplete decline in CYFRA predicted poorer outcome.
Nisman et al[13]	Advanced	Declines of CYFRA 21-1 levels of ⩾35% after two cycles of chemotherapy was a reliable marker for treatment efficacy and survival.
Any stage		
Karnak et al[14]	Any	CYFRA levels had a sensitivity of 65% for NSCLC, 71% for squamous cell, and 46% for adenocarcinoma. Sensitivity ranged from stage I at 38% to stage III at 87.5%. Specificity for all stages was 92%.
Kulpa et al[15]	Any	CYFRA was significantly higher in advanced than early-stage disease and an independent prognostic marker in early disease.
Hatzakis et al[16]	Any	CYFRA and NSE are the most useful markers in differentiating cell type. When measured at diagnosis, CYFRA may provide prognostic information.
Lee and Chang[17]	Any	In diagnosing malignant pleural effusions, CEA was the most prognostic tumor marker. CYFRA in pleural fluid was 61% sensitive and 81% specific.
Buccheri et al[18]	Any	Use of serum cytokine markers before, during, and after treatment should be completed to access status of disease and response to treatment. There is no preference between CYFRA and tissue polypeptide antigen.
Hillas et al[19]	Any	CYFRA levels of induced sputum samples had a 86% sensitivity, 75% specificity, 88% positive predictive value, and 72% negative predictive value for cancer diagnosis.

RA NSCLC, non–small-cell lung cancer; OS, overall survival; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen.

Durham, NC—Jeffrey Crawford, MD, supported by CA47577; Georgetown University Medical Center, Washington, DC—Minetta C. Liu, MD, supported by CA77597; Cancer Centers of the Carolinas, Greenville, SC—Jeffrey K. Giguere, MD, supported by CA29165; Hematology-Oncology Associates of Central New York CCOP, Syracuse, NY—Jeffrey Kirshner, MD, supported by CA45389; Nevada Cancer Research Foundation CCOP, Las Vegas, NV—John A. Ellerton, MD, supported by CA35421; New Hampshire Oncology-Hematology PA, Concord, NH—Douglas J. Weckstein; Rhode Island Hospital, Providence, RI—William Sikov, MD, supported by CA08025; Roswell Park Cancer Institute, Buffalo, NY—Ellis Levine, MD, supported by CA59518; Southeast Cancer Control Consortium Inc. CCOP, Goldsboro, NC—James N. Atkins, MD, supported by CA45808; State University of New York Upstate Medical University, Syracuse, NY—Stephen L. Graziano, MD, supported by CA21060; University of California at San Diego, San Diego, CA—Barbara A. Parker, MD, supported by CA11789; University of California at San Francisco, San Francisco, CA—Charles J. Ryan, MD, supported by CA60138; University of Chicago, Chicago, IL—Hedy L. Kindler, MD, supported by CA41287; University of Iowa, Iowa City, IA—Daniel A. Vaena, MD, supported by CA47642; University of Maryland Greenebaum Cancer Center, Baltimore, MD—Martin Edelman, MD, supported by CA31983; University of Minnesota, Minneapolis, MN—Bruce A. Peterson, MD, supported by CA16450; University of Missouri/Ellis Fischel Cancer Center, Columbia, MO—Michael C. Perry, MD, supported by CA12046; University of North Carolina at Chapel Hill, Chapel Hill, NC—Thomas C. Shea, MD, supported by CA47559; University of Texas Southwestern Medical Center, Dallas, TX—Debasish Tripathy, MD; University of Vermont, Burlington, VT—Steven M. Grunberg, MD, supported by CA77406; and Washington University School of Medicine, St. Louis, MO—Nancy Bartlett, MD, supported by CA77440.

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